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MOLTING HORMONE-LIKE SUBSTANCES FROM *AJUGA IVA* (LABIATAE), ISOLATION AND SPECTRAL IDENTIFICATION

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ABSTRACT

Two phytoecdysteroids were isolated chromatographically from *Ajuga iva* (Labiatae) growing in Jordan. The isolated components were identified as C-29 cyasterone (I); and C-27 ecdysterone (II). The weight percentage recoveries on an air-dry basis of I and II without optimization were found to be 0.03% and 0.02% (w/w), respectively. Structural elucidation of the titled compounds was based on their elemental analysis and spectral data viz. MS, ^{1}H-NMR, IR, and UV.

KEY WORDS: phytoecdysteroids, molting hormones, cyasterone, ecdysterone, *Ajuga iva*, Labiatae

INTRODUCTION

The natural occurrence of bioactive materials in plants warrants a basic research as it suggests the possibility of its exploitation in the medicinal field and/or pest management (Williams 1967; Berkoff 1971; Bowers & Nishida 1980; Saxe 1987; Aranson 1989; Beier 1990). Additionally, the knowledge of the structure of bioactive chemicals makes synthesis of similar or related derivatives quite possible (Jones *et al.* 1986), taking into consideration that synthesis of anthropogenics without guidance is tedious and quite involved.

Ajuga iva is an herbaceous plant grown widely in Jordan, and known there as Ja'da. The extract of this plant is used traditionally as a diuretic, cardiac tonic, or to cure fever and sore throat (Hikino *et al.* 1968; Kubo *et al.* 1982). Several authors (Hikino *et al.* 1968; Kubo *et al.* 1982; Miller *et al.* 1985; Jones *et al.* 1986; Saxe 1987) reported that this plant exhibited antifeedant and insect ecdysis properties, as well as the fact that it has activity against hypercholesterolemia and hyperglyceridemia.

Moreover, it has been reported that *Ajuga* extract exhibits a high stimulating effect on protein synthesis in animals (Robbins *et al.* 1970; Karel 1972).

Reviewing literature on this subject, it was noticed that several bioactive chemicals including clerodane diterpenoid, ajugarines, C-28 ecdysterone, and other ecdysones were isolated from various species of *Ajuga* such as *A. chia* (Poir.) Schreb., *A. iva, A. remota* Benth., *A. nipponensis* Makino, and *A. orientalis* L. grown in various parts of the world. However, nothing in the literature could be traced with the phytochemistry of *Ajuga iva* growing in Jordan. Since taxonomy and environmental conditions can play a significant role in variations of plant constituents, it was deemed relevant to investigate this plant as a source of bioactive material that may find a useful application. The search in this field included isolation and spectral identification of the principal constituents of the entire plant.

EXPERIMENTAL

Melting points were not corrected, ^{1}H-NMR spectra were recorded on a Varian HA-100 spectrometer in C_5D_5N. Chemical shifts were expressed in ppm downfield from TMS as an internal standard. Abbreviations: s = singlet; d = doublet. ^{1}H-NMR and microanalysis were carried out by research laboratories at Birmingham University. Electron impact mass spectra were carried out by research laboratories at Nottingham University. IR spectra in KBr were run on a Perkin-Elmer S-21 spectrophotometer. All chemicals and solvents were of HPLC or analytical grade and were used as such.

EXTRACTION AND ISOLATION

The whole fresh plant material was collected locally from north Jordan, identified, washed under running water, air dried in the shade, and ground into small pieces. The entire powdered plant (2 kg) was suspended in methanol and homogenized at ambient temperature according to percolation rules. The combined filtered extract was then concentrated under vacuum and treated with water until 30% (v/v) aqueous methanol was obtained. The final solution was repeatedly extracted with hexane to remove chlorophyll and non-polar coextractants. The mother liquor was then concentrated at reduced pressure to about half its volume and extracted five times with ethyl acetate. The green organic layer was evaporated under vacuum almost to dryness, and finally chromatographed on a silica gel column. Elution was made with $CHCl_3$:MeOH (9:1) with increasing polarity to a ratio of 4:1. Fractions were screened by thin layer chromatography (TLC) in hexane/acetone, and combined together according to their TLC pattern, resulting in the isolation of two crude components; I and II. Yields from the entire plant and without optimization of I and II were 0.03 and 0.02% respectively.

IDENTIFICATION

Crystallization of compound I from MeOH:$CHCl_3$ furnished colorless needles (m.p. 162-164°C), and produced one spot with pink color--positive for steroidal compounds as tested with Sonnenshein's spray reagent on the TLC plate (Karel 1972). Elemental analysis found: C 64.6; H 8.4; O 27.0--required for $C_{29}H_{44}O_8$ (C 64.7; H 8.6; O 26.7). Mass spectrometry produced m/z 520 $M^{+\bullet}$ as a very weak peak relative to the base line at m/z 43. ^{1}H-NMR (Table 1) matches literature values for cyasterone (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a). Acetylation of I in the usual manner (Ac_2O in pyridine at room temperature) gave the respective 2,3,22-tri-acetate, m.p. 251-252°C (elemental analysis found: C 64.7; H 7.5 required for $C_{35}H_{50}O_{11}$ [C 64.99; H 7.8]).

Crystallization of compound II from MeOH:$CHCl_3$ gave a white solid, m.p. 243-245°C and showed one spot with brown color--positive for steroidal compounds as tested with Sonnenshein's spray reagent on the TLC plate. Elemental analysis found: C 67.29; H 9.13; O 23.58--required for $C_{27}H_{44}O_6$ (C 67.47; H 9.23; O 23.30). ^{1}H-NMR (Table 1) matches literature values for ecdysterone (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a, 1971b; Sabri *et al.* 1981; Miller *et al.* 1985).

RESULTS AND DISCUSSION

In the course of our investigation of *Ajuga iva* growing in Jordan as a source of bioactive materials, we found that the crude methanol extract of the whole plant contained two phytoecdysteroids, cyasterone--designated I; and ecdysterone or 20-hydroxyecdysone--designated II (Figure 1). The percentage occurrences of the two chemicals without optimization were estimated as 0.03 and 0.02%, respectively.

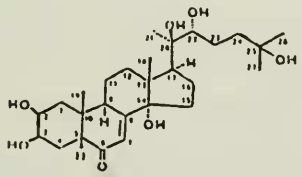
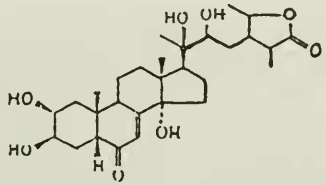


Fig. 1. Cyasteron, I



Ecdysterone, II

Previous studies in this field revealed that various species of *Ajuga* (e.g., *A. chia*, *A. iva*, *A. remota*, and *A. nipponensis*) contain several constituents including clerodane diterpenoid (Camps *et al.* 1982; Jones *et al.* 1986); ajugarins (Shimomura *et al.* 1981; Kubo *et al.* 1982); C-28 ecdysone (Khafagy & Sabri 1979); and steroidal molting hormone-like substances (ecdysones) (Ikan & Ravid 1971a; Sabri *et al.* 1981). In our study, we found that the whole plant of *Ajuga iva* growing in Jordan contains two major ecdysterones; *i.e.*, cyasterone (I), the principal sterol in animals; and ecdysterone (II), the main sterol in plants. In this context, it is worth mentioning that variation among constituents comes as a consequence of species and environmental condition variations.

The structure elucidation of the designated chemicals has been deduced from their elemental analysis and spectral data, as compared with the matched literature values. Elemental analysis of cyasterone was in full agreement with the proposed formula.

The mass spectrum of compound I revealed the correct molecular ion as a very weak peak at m/z (520, $M^{+\cdot}$) relative to the base line at m/z 43. The principal electron impact fragmentations were in good accordance with the assigned structure and were rationalized as follows: peaks at m/z (484, 466, 448, and 430) corresponding to stepwise losses of one to five molecules of water from the parent molecular ion, [$M^{+\cdot}$-(1-5) H_2O]. The peaks at m/z (505, 487, 469, 451, and 433) might be attributed to a fission of one methyl group from the parent ion followed by subsequent losses of one to five molecules of water from [M-157]$^{+\cdot}$. The rupture of the C_{17}-C_{20} bond followed by hydrogen transfer and loss of water molecules could be the source of the ion at m/z 300. Finally, the fragment at m/z 201 disclosed a C_{17}-C_{20} bond cleavage without rearrangement.

In further support of the suggested cyasterone structure, acetylation of compound I gave the respective 2,3,22-tri-acetate with m.p. and elemental analysis in full agreement with literature values. ^{1}H-NMR spectral data for compound I and its tri-acetate derivative (Table 1), all were in very close similarity to the steroidal nucleus. However, variations occurred in the side chain. It is worth mentioning here that the characteristic bands for acetyl groups on carbons 2, 3, and 22 appeared as singlets at 2, 2.11, and 2.14 ppm. However, protons of carbons 2, 3, 9, 22, and 29 appeared as multiplets down from TMS at 5.02, 5.32, 3.12, 4.08, and 4.1 ppm, respectively.

Table 1. ^{1}H-NMR of *Ajuga* phytoecdysterones in C_5D_5N. Chemical shifts at δ (ppm) from internal TMS. (s = singlet, d = doublet)

	Proton assignments of Carbon:						
Compounds	18	19	21	26	27	29	7
Cyasterone	1.24(s)	1.08(s)	1.57(s)	--	1.34(d)	1.34(d)	6.23(d)
Cyas-triacetate	0.86(s)	1.03(s)	1.25(s)	--	1.30(d)	1.41(d)	5.86(d)
Ecdysterone	1.19(s)	1.05(s)	1.55(s)	1.35(s)	1.35(s)	--	6.2(d)

The characteristic IR bands (cm^{-1}) for compound I at 3480, 1174, and 1618 were assigned for hydroxyl, γ-lactone, and enone systems. The IR bands and the UV absorbing maxima at 243 ($\log\epsilon$ = 4.1) together with ^{1}H-NMR singlet at δ = 6.23 ppm, all confirm the proposed C_{29}-cyasterone.

The mass spectrum for compound II did not show the molecular ion. However, the remainder of mass fragmentations together with the m.p., elemental analysis and ^{1}H-NMR data were in full agreement with literature data (Hikino *et al.* 1968; Imai *et al.* 1969; Ikan & Ravid 1971a, 1971b; Mandava 1985; Miller *et al.* 1985) for ecdysterone. The IR and UV spectra were made as a further proof of the assigned structure. ν^{KBr} (cm^{-1}) at 3400, 1650, and 1630 were assigned to hydroxyl, carbonyl, and olefinic groups, respectively. The final IR bands and the UV absorbing maxima at 343 together with the ^{1}H-NMR singlet at δ(6.2 ppm) exhibited by compound II, all confirm the α,β-unsaturated ketone system, and in full accordance with the assigned C_{27}-ecdysterone.

Finally, it is anticipated in this context that a question will arise as to whether the insecticidal potential of the isolated materials was monitored. In reality, the amount dealt with and objectives stated were not geared for this purpose. However, the isolated chemicals are not without precedents. Several authors (Galbraith & Horn 1966; Williams & Salami 1966; Williams 1967; Hikino *et al.* 1968; Ikan & Ravid 1971b; Karel 1972; Mandava 1985) reported that ecdysones and/or molting hormone-like substances from plants mediate several aspects of larval development, its presence over the entire life cycle forces larvae to develop abnormally or fail to mature; and hence it has been suggested that plants synthesize such types of compounds as a self defense against insect attack (Galbraith & Horn 1966; Williams 1967; Mandava 1985), with the advantage that are not only specific but also proof against evolution of resistance (Williams 1967).

In conclusion, two phytoecdysterones, viz. cyasterone and ecdysterone have been isolated and identified from *Ajuga iva* grown in Jordan. In regard to the usefulness, we suggest the possibility of its exploitation in an integrated pest management (IPM) program, provided that the detrimental effect on the environment including man, is assessed prior to its application.

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